

U.S.S.N. 09/096,648

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AMENDMENT

06  
allowing the embryo to develop into a transgenic non-human mammal comprising a satellite artificial chromosome.

#### REMARKS

A check for the fee for a three month extension of time accompanies this response. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 50-1213.

It is noted that a Change of Address has been filed in connection with this application. Please send all correspondence for this application to the new address as set forth in the Change of Address and as shown below in this correspondence.

Claims 32-39, 43, 44, 59, 60, 65, 67, 71-74, 82-89 and 93-100 are presently pending in this application. Claims 40, 41 and 90-92 have been cancelled without prejudice. Claims 32, 33, 43, 44, 71, 73, 74, 93 and 95-99 have been amended in order to more particularly point out and distinctly claim the subject matter that applicant regards as the invention. No amendments have been made to obviate prior art and no new matter has been introduced. The amendments to claims 32, 33, 43, 44, 71, 73, 74, 93 and 95-99 find basis in the specification and claims as originally filed. Therefore, since the amendments change the form, not the substance of the claimed subject matter, no new matter has been added. Accordingly, entry of the amendments to the claims is respectfully requested.

A marked up copy per 37 C.F.R. §1.121 of the amended claims is attached to this response.

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**THE REJECTION OF CLAIMS 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-105  
UNDER 35 U.S.C. §112, FIRST PARAGRAPH**

Claims 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-105 are directed to methods of producing a transgenic animal or transgenic embryo and to non-human transgenic embryos. Independent method claims 32, 43, 44, 73, 74, 90, 93, 95 and 96 include steps of introducing a cell comprising an artificial chromosome into a female animal and allowing the cell to develop into a transgenic animal comprising an artificial chromosome. Independent method claim 82 includes steps of introducing a satellite artificial chromosome into a cell and culturing the cell under conditions whereby it develops into an embryo. Independent method claims 97, 98 and 99 include steps of introducing an embryo, fertilized oocyte or mouse embryonic stem cell, respectively, comprising a satellite artificial chromosome into a female animal and allowing it to develop into a transgenic animal comprising a satellite artificial chromosome. Independent product claims 101-105 are directed to a non-human transgenic embryo comprising either a satellite artificial chromosome or an artificial chromosome produced by a specified process.

It is acknowledged in the Office Action that the Perez Declaration under 37 C.F.R. §1.132 filed February 1, 2001 (Paper No. 17), in response to the previous Office Action (Paper No. 12 mailed June 21, 2000) is sufficient with regard to the following scope: a method for producing a transgenic non-human mammal comprising introducing into a female non-human mammal an ovum comprising a SATAC, wherein the ovum is fertilized into a zygote or embryo; and allowing the zygote or embryo to develop into a transgenic non-human mammal comprising the SATAC; and a method for producing a transgenic mouse comprising introducing mouse embryonic stem cells comprising a SATAC into a mouse embryo and introducing the embryo into a female mouse; and allowing the embryo to develop into a transgenic mouse comprising the SATAC.

Although the Perez Declaration is acknowledged as sufficient with regard

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to the scope recited in the Office Action, each of claims 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-105 is rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in pages 2-7 of the prior Office Action mailed June 21, 2000 (Paper No. 12). Specifically, it is stated that all claims are included in the rejection because certain of the claims are still directed to non-human embryonic stem cells or are incomplete because the claims are missing critical steps depending upon the source "cell(s)". With respect to claims directed to non-human transgenic embryos, it is alleged that although the Perez Declaration supports the production of transgenic mice and bovine embryos using SATACs and confirms the expression of detectable marker genes, it fails to teach how to use the mice as expression fails to convey a useful phenotype as a result of expression. It is concluded in the Office Action that the specification fails to teach both how to make and how to use the claimed invention because, in light of the specification, the claimed invention is properly interpreted with regard to the disclosed use of the claimed transgenic "animals" comprising SATACs for conveying a state of disease resistance.

Reconsideration of these grounds for the rejection is respectfully requested based on the following remarks.

**Rebuttal of the specific issues raised in the office action.**

**Claim scope acknowledged as enabled in the Office Action**

It is acknowledged in the Office Action that claims directed to methods of producing a transgenic animal are enabled with respect to the production of transgenic non-human mammals via a process that includes introduction of a particular cell type into a female non-human mammal which is allowed to develop into a transgenic non-human mammal. Each of the claims directed to a method of producing a transgenic animal has been amended herein to specify that the method is for production of transgenic non-human mammals using a female non-human mammal and allowing for the development of a transgenic non-human mammal. Accordingly, it is respectfully submitted that such claims

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are commensurate in scope with that deemed enabled by the examiner in the Office Action with respect to the types of animals that are used and produced by these methods.

**Claims directed to methods of producing transgenic animals which specify use of embryonic stem cells**

It is alleged in the Office Action that only embryonic stem cells isolated from the mouse had been established in the art at the time of effective filing of the instant application, yet there are claims directed to "embryonic stem cells" which are not limited to "mouse embryonic stem cells." It is further noted in the Office Action that "Applicants indicate in the Response on page 27 that the claims have been amended to overcome this aspect of the enablement rejection," however "see claims 32, 33, 37, 43, 44, 73, etc., which have not been so limited."

Applicant notes that of the claims thus cited in the Office Action (i.e., "claims 32, 33, 37, 43, 44, 73, etc."), only claim 33 specifically recites the phrase "stem cell". Furthermore, of all the pending claims, only claims 33, 71, 87 and 99 specifically recite the phrase "stem cell," and, in claims 87 and 99, it is specified that the stem cell is a mouse embryonic stem cell. Because most of the claims cited in the Office Action in the context of this particular rejection do not recite the phrase "stem cell," it is not clear why such claims (i.e., "claims 32, 37, 43, 44, 73, etc.") are included in the rejection. Lack of a reference to "stem cells" in most of the claims makes it nonsensical to "limit the claims to mouse embryonic stem cells."

Because claims 87 and 99 specify that the stem cells are mouse cells, it appears that there is no basis for rejecting these claims on an allegation that stem cells from mice were the only stem cells established at the time of filing of the instant application. With respect to claims 33 and 71, in the interest of advancing prosecution, the claims have been amended to specify "mouse" stem cells, thereby rendering moot any such rejection.

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**Claims directed to methods of producing transgenic animals which recite "cell(s)" or specify particular cells**

It is alleged in the Office Action that claims that recite "cell(s)" or specify certain cell types (other than embryonic stem cells) are incomplete because they are missing critical steps depending upon the source cells. As an example, the Office Action refers to an unfertilized oocyte and a germ cell that is not a fertilized ovum, zygote or embryo, as cells that will not develop into an animal. As set forth in the following discussion, Applicant respectfully disagrees with these grounds for rejection of the claimed subject matter under 35 U.S.C. §112, first paragraph.

Of the independent method claims that will be pending upon entry of the amendments herein, claims 32, 43, 44, 73, 74, 82, 93, 95 and 96 are directed to methods of producing a transgenic animal or embryo comprising steps of introducing a cell comprising an artificial chromosome into a female animal and allowing the cell to develop into a transgenic animal or embryo. The remaining of the pending independent method claims (i.e., claims 88, 97, 98 and 99) specify that the cell comprising an artificial chromosome is a particular type of cell (i.e., embryo, fertilized oocyte or mouse embryonic stem cell). It is respectfully submitted that, contrary to the assertion in the Office Action, each of the claims directed to a method of producing a transgenic animal contains all essential steps in the method and is therefore complete.

A variety of cells may be used in the claimed methods. Exemplary cells for use in the claimed methods are referred to in the application. For example, the specification refers to procedures in which exogenous genetic material is introduced into a pronucleus of a mammalian zygote or fertilized oocyte by microinjection [see, e.g., U.S. Patent Nos. 4,873,191 and 5,354,674; see, also, International PCT application No. WO95/14769, which is based on U.S. application Serial No. 08/159,084] and the resulting embryo or zygote is transplanted into a host female uterus and allowed to develop.

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In addition, in considering the teachings of the specification in combination with transgenic animal production methods known in the art at the time of filing of the instant application, it is clear that one of skill in the art could readily determine a number of cells that may be used in the claimed methods. For example, nuclear transfer methods for generating transgenic animals were known at the time of filing of the instant application [see, e.g., Campbell *et al.* (1996) *Nature* 380:64-66 and WO95/17500]. In these methods, a donor nucleus, which may contain heterologous nucleic acid, such as a transgene, is transferred into an enucleated oocyte which is then transferred into a recipient female for development into a transgenic animal. Thus, in these methods, an unfertilized oocyte does in fact develop into an animal, contrary to the assertion in the Office Action that such a cell will not develop into an animal. It is respectfully submitted, therefore, that fertilization is not essential to the claimed methods, and the pending method claims that recite "cell(s)" (i.e., claims 32, 43, 44, 73, 74, 82, 93, 95 and 96) are thus not missing critical steps depending upon the source cells but instead are complete as written. Furthermore, for this reason, dependent method claims (claims 35, 36, 59, 60, 67, 72, 83, 84, 93, 94 and 100) that either do not specify a cell type or specify the type of cell that is introduced into a female animal (or that is cultured to develop into an embryo) but that do not refer to fertilization are also complete.

It is further submitted that the rejection of the claimed methods as allegedly incomplete also clearly does not apply to the independent method claims (i.e., claims 88, 97, 98 and 99) that specify that the cell comprising an artificial chromosome is a particular type of cell. In claim 88, the cell into which a satellite artificial chromosome is introduced is an embryo. In claims 97, 98 and 99, the cell that is introduced into a female animal is an embryo, fertilized oocyte and embryo, respectively. Each is capable of development into an embryo or transgenic animal, and the claims are thus complete. Similarly, it is respectfully submitted that the rejection does not apply to dependent claims

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(claims 34, 37, 38, 39, 65, 71, 85, 86, 87 and 89) that specify that the cell comprising an artificial chromosome is a particular type of cell (e.g., an embryo, zygote, fertilized ovum, germline cell or embryonic stem cell) that is capable of developing into an animal or embryo or that specify that the artificial chromosome is contained within a pronucleus.

**Claims directed to transgenic embryos (claims 101-105)**

It is alleged in the Office Action that claims directed to a transgenic embryo are not enabled because claims are to be given their broadest reasonable interpretation that is consistent with the specification, which, in the instant application, discusses use of SATACs in producing disease-resistant transgenic animals, and the specification, as well as evidence presented in the Perez Declaration, fail to support that expression of any gene would result in immunoprotection.

In the interest of advancing prosecution, the rejected claims directed to transgenic embryos have been canceled thereby rendering any rejection based on this allegation moot. Applicant does not concede that the basis given in the Office Action for this rejection is proper, and the cancelled claims will be pursued in a continuing application.

**THE REJECTION OF CLAIMS 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-100 UNDER 35 U.S.C. §112, SECOND PARAGRAPH**

All claims directed to a method of producing a transgenic animal or embryo (i.e, claims 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-100) are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, it is alleged in the Office Action that the claims are incomplete because they are missing critical steps depending upon the source cells. The Office Action makes directs applicant's attention to "identified enabled scope above." Thus, it appears that the same rejection of

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the claims is being made under 35 U.S.C. §112, first paragraph, as under 35 U.S.C. §112, second paragraph.

For the reasons provided above with respect to the rejection of the method claims on the same basis under 35 U.S.C. §112, first paragraph, it is respectfully submitted that all of the essential steps of the claimed methods are provided in the claims as pending. Accordingly, the rejection of the claims as indefinite due to a lack of completeness is respectfully traversed.

**THE REJECTION OF CLAIMS 32-41, 43, 44, 65, 71, 72 and 82-105 UNDER 35 U.S.C. §102(e)**

Claims 32-41, 43, 44, 65, 71, 72 and 82-105 are rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Scheffler (U.S. Patent No. 6,133,503) because Scheffler teaches the production of transgenic mice using MACs and the methods and mice of Scheffler meet all of the limitations of the claimed invention, particularly since the MACs of Scheffler meet the description of SATACs of the instant invention (page 6, lines 6-23 of the specification). This rejection 35 U.S.C. §102(e), with respect to any of the pending cited claims, is respectfully traversed.

**Relevant Law**

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference.

Lindemann Maschinen-fabrik GmbH v. American Hoist and Derrick Co., 730 F.2d

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1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

**The claims**

Claims 32-41, 65, 82-89, 93-95 and 97-100 are directed to methods of producing transgenic animals or embryos, each of which includes a step of introducing a cell comprising a satellite artificial chromosome into a female animal or introducing a satellite artificial chromosome into a cell. Claims 43, 44, 71, 72 and 96 are directed to methods of producing transgenic animals, each of which includes a step of introducing a cell comprising a minichromosome comprising a neo-centromere or a cell comprising an artificial chromosome that comprises more heterochromatic than euchromatic nucleic acid into a female animal. Claims 90-92 and 101-105 have been cancelled herein; accordingly, the rejection of these claims under 35 U.S.C. §102(e) is moot with respect to these claims.

**The cited art - Scheffler (U.S. Patent No. 6,133,503)**

Scheffler discloses methods of making mammalian artificial chromosomes (MACs) by fragmenting a parental chromosome and selecting a centromeric fragment of the chromosome containing less than about 0.1% of the DNA present in a normal haploid genome containing the parental chromosome (see column 13, lines 36-40). Fragmentation may be accomplished by, for example, telomere associated chromosome truncation or irradiation of a cell (see column 14, lines 8-13). The MACs are prepared by obtaining a centromeric fragment of a chromosome containing a selectable marker which can be an endogenous gene located near the centromere on the chromosome fragment or can be randomly or site-specifically inserted into the pericentric region of a chromosome

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by homologous recombination into a gene that is located in a pericentric region of a chromosome (column 14, lines 19-41).

The MACs thus produced by fragmentation of existing normal cellular chromosomes are described as being defined, in part, by having a size, excluding the centromere, that is less than about 0.1% of DNA present in a normal mammalian haploid genome. Thus, the MACs contain a centromere and less than about 3.3 million base pairs of DNA (column 4, lines 15-45). The MACs are also characterized as containing a unique cloning site (column 6, lines 26-27). Scheffler also discloses that the MACs are useful for producing a transgenic animal expressing a gene of interest and provides particular methods for producing such transgenic animals (column 18, lines 37-58).

**Differences between the claimed methods of producing transgenic animals or embryos and Scheffler**

The claimed methods for the production of transgenic animals or embryos utilize distinct and specific artificial chromosomes that are not disclosed by Scheffler: satellite artificial chromosomes, or an artificial chromosome that comprises more heterochromatic than euchromatic nucleic acid, and minichromosomes containing a neo-centromere. As described in the specification, satellite artificial chromosomes and minichromosomes used in the claimed methods possess a unique architecture based on an array of repeated amplicons, each of which can contain multiple copies of heterologous DNA. Satellite artificial chromosomes and minichromosomes of the claimed methods are, however, distinct structures. Satellite artificial chromosomes are substantially all heterochromatin, except for portions of heterologous DNA that has been introduced into the structure.

Minichromosomes utilized in the claimed methods are distinct structures from satellite artificial chromosomes that are formed *de novo*. As described in the specification (see, for example, page 21, line 14, through page 22, line 11), the chromosomes of the claimed methods result from amplification of

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chromosomal and heterologous DNA to yield a dicentric chromosome containing a new additional centromere (i.e., the neo-centromere). Separation of the dicentric chromosome between the two centromeres yields a neo-minichromosome containing the neo-centromere and the multiple copies of the heterologous DNA. As described on page 49, lines 1-24, of the specification, the neo-minichromosome possesses a unique architecture wherein the arm of the minichromosome is made up of repeated units that contain multiple copies of the heterologous DNA together with some of the chromosomal DNA. The unique structure of the *de novo*-formed neo-minichromosome is of a defined composition, i.e., repeat-after-repeat of the heterologous DNA-containing unit, which provides for enhanced levels of expression of any heterologous gene contained therein.

The specification further distinguishes satellite artificial chromosomes and the minichromosomes containing a neo-centromere with respect to methods of formation of each. Specifically, the specification describes that a formerly dicentric chromosome is a chromosome that is produced when a dicentric chromosome fragments and acquires new telomeres so that two chromosomes, each having one of the centromeres, are produced. Each of the fragments, are replicable chromosomes. If one of the chromosomes undergoes amplification of euchromatic DNA to produce a full functionally chromosome that contains the heterologous DNA and primarily [at least more than 50%] euchromatin, it is a minichromosome. The remaining chromosome is a formerly dicentric chromosome. If one of the chromosomes undergoes amplification, whereby heterochromatin [satellite DNA] is amplified, a euchromatic portion [or arm remains], it is referred to as a sausage chromosome. A chromosome that is substantially all heterochromatin, except for portions of heterologous DNA, is called a SATAC (satellite artificial chromosome). Such chromosomes (SATACs) can be produced from sausage chromosomes by culturing the cell containing the sausage chromosome under conditions, such as, e.g., BrdU treatment and/or

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growth under selective conditions, that destabilize the chromosome so that a satellite artificial chromosome is produced. SATACs may not necessarily be produced in multiple steps, but may appear after the initial introduction of the heterologous DNA and growth under selective conditions, or they may appear after several cycles of growth under selective conditions and BrdU treatment.

In contrast, the MAC disclosed in Scheffler is a small portion of an existing chromosome produced by fragmenting a normal cellular chromosome. For example, telomere associated chromosome truncation is used to make the MACs and is a process that requires the introduction of a very specific exogenous DNA sequence, i.e., telomeric sequence, into an existing chromosome. In this method of telomere-associated chromosome fragmentation, a terminal portion of the existing chromosome is simply broken off from the chromosome and replaced with the added telomeric sequence, thereby reducing the size of the existing chromosome. The smaller-size chromosome still contains the original centromere and a large portion of the original heterogeneous chromosomal DNA of unknown composition.

The artificial chromosomes specified in the claimed methods are not simply unmodified fragments of existing chromosomes. They are produced by processes, which are described and illustrated in great detail in the instant application, wherein amplification of a chromosome generates new chromosomes with distinct structures based in repeating units containing multiple copies of heterologous DNA. These processes and structures are not taught or suggested by Scheffler.

There is no suggestion in Scheffler of a method for generating a new centromere or a chromosome containing a new centromere and a heterologous DNA repeat unit-based structure. There is no suggestion in Scheffler of selecting minichromosomes containing a neo-centromere or multiple repeats of heterologous DNA-containing units or of a satellite artificial chromosome containing more heterochromatic than euchromatic DNA because simple

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fragmentation of an existing chromosome fragmentation as disclosed in Scheffler does not produce a minichromosome containing a neo-centromere or a satellite artificial chromosome. As such, the instantly claimed methods are not taught or suggested by Scheffler.

**THE REJECTION OF CLAIMS 32, 67, 73 and 74 UNDER 35 U.S.C. §103(a)**

Claims 32, 67, 73 and 74 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Scheffler (U.S. Patent No. 6,133,503) taken with Hadlaczky (U.S. Patent No. 5,288,625). Specifically, it is alleged that although Scheffler differs from the claimed methods in that it does not teach the specific deposited cell lines (EC3/7C5 and KE1 2/4) and/or methods of generating cell hybrids using the deposited cell lines, Hadlaczky teaches the formation of stable chromosomes derived from the deposited cell lines. It is concluded in the Office Action that in view of the teachings of Hadlaczky, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the methods of Scheffler of producing transgenic mice or non-human mammals by utilizing the stable chromosomes generated by the cell lines of Hadlaczky. The rejection under 35 U.S.C. §103(a) is respectfully traversed.

**Relevant law**

In order to set forth a prima facie case of obviousness under 35 U.S.C. §103, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. See, e.g., Lindemann Maschinen-fabrik GmbH v. American Hoist and Derrick Co., 730 F.2d 1452, 1462, 221 USPQ 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re

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Fritch 23 USPQ 1780 (CAFC 1992); see, also, In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

**The claims**

Claim 32 is directed to a method of producing a transgenic non-human mammal that includes a step of introducing a cell comprising a satellite artificial chromosome into a female non-human mammal. Claim 67, which is dependent on claim 32, specifies that the satellite artificial chromosome is a megachromosome derived from a cell line having all of the identifying characteristics of the cell line deposited under ECACC accession no. 96040928 or 96040929. Claims 73 and 74 are directed to methods of producing a transgenic non-human mammal that include steps of introducing DNA encoding a gene product(s) into a cell containing the minichromosome of cell line EC3/7C5 or the  $\lambda$  neo-chromosome of cell line KE1 2/4, growing the cells under conditions whereby minichromosomes or  $\lambda$  neo-chromosomes containing the DNA encoding a gene product(s) are produced, isolating such minichromosomes or  $\lambda$  neo-chromosomes, introducing them into an animal cell which is introduced into a female non-human mammal and allowing the cell to develop into a transgenic non-human mammal.

**Differences between the claims and the teachings of the cited references**

As discussed above with reference to the rejection under 35 U.S.C. §102(e), Scheffler discloses methods of making mammalian artificial chromosomes (MACs) by fragmenting a parental chromosome and selecting a centromeric fragment of the chromosome (i.e., a MAC) containing less than about 0.1% of the DNA present in a normal haploid genome containing the parental chromosome. Scheffler further teaches that the MACs are useful for producing a transgenic animal expressing a gene of interest and provides particular methods for producing such transgenic animals.

As also discussed above, Scheffler does not teach or suggest a method for generating a new centromere or a chromosome containing a new centromere

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and a heterologous DNA repeat unit-based structure. There is no suggestion in Scheffler of selecting minichromosomes containing a neo-centromere or multiple repeats of heterologous DNA-containing units or of a satellite artificial chromosome or an artificial chromosome containing more heterochromatic than euchromatic DNA as taught in the instant application. In particular, there is no suggestion in Scheffler of cell lines containing satellite artificial chromosomes, such as cell lines deposited under ECACC accession no. 96040928 or 96040929, or cell lines containing minichromosomes or  $\lambda$  neo-chromosomes, such as the EC3/7C5 or KE1 2/4 cell lines.

Hadlaczky (U.S. Patent No. 5,288,625) discloses cell lines EC3/7C5 and EC3/7C6) which carry an extra centromere (the neo-centromere) solely on minichromosomes. Hadlaczky also discloses hybrid cell line KE1 2/4, formed by fusing EC3/7 mouse cells with CHO K-20 hamster cells, in which a new centromere is found on a new chromosome, i.e., the  $\lambda$  neo-chromosome, which has the size of an average mouse chromosome. Hadlaczky suggests that the  $\lambda$  neo-chromosome was formed by an extensive amplification process.

There is no teaching or suggestion in Hadlaczky of a satellite artificial chromosome. The teachings of Hadlaczky are limited to production and analysis of the neo-minichromosome and  $\lambda$  neo-chromosome. Furthermore, Hadlaczky fails to teach or suggest stable integration of heterologous DNA into the neo-minichromosome or  $\lambda$  neo-chromosome. The instant application, however, teaches that the neo-minichromosome contains megabases of  $\lambda$  DNA sequences that can serve as target sites for homologous recombination and integration of desired gene(s) into the neo-minichromosome and that the minichromosome and  $\lambda$  neo-chromosome may be used for carrying large fragments of heterologous DNA and thus as vectors for genetic engineering of cells, such as in the development of transgenic animals.

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**The combination of Scheffler and Hadlaczky does not result in the instantly claimed methods**

The combination of the teachings of Scheffler and Hadlaczky fail to yield the instantly claimed methods. With respect to claims 32 and 67, neither of the cited references, singly or in combination, suggests the satellite artificial chromosomes that are utilized in steps of the claimed method.

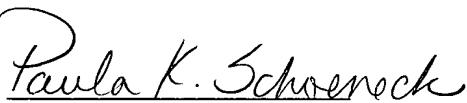
With respect to claims 73 and 74, Scheffler fails to teach minichromosomes or λ neo-chromosomes containing a neo-centromere, and in particular fails to teach the specific cell lines recited in the claims. Although Hadlaczky discloses the specific cell lines EC3/7C5 and KE1 2/4, it fails to disclose that the minichromosomes or λ neo-chromosomes contained in the cells may be used for integration of foreign transgenes as specified in claims 73 and 74. Therefore, because the combination of references fails to arrive at the claimed methods, they cannot deprive the claims of patentability under 35 U.S.C. §103(a).

\* \* \*

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,  
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Hadlaczky *et al.*

Serial No.: 09/096,648

Filed: June 12, 1998

For: *ARTIFICIAL CHROMOSOMES,  
USES THEREOF AND METHODS  
FOR PREPARING ARTIFICIAL  
CHROMOSOMES*

Art Unit: 1632

Examiner: Martin, J.

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:  
Commissioner for Patents  
Washington, D.C. 20231, on this date.

09/04/01  
Date

  
Paula Schoeneck

**MARKED UP CLAIMS (37 C.F.R. § 1.121)**

Please amend claims 32, 33, 43, 44, 71, 73, 74, 93 and 95-99 as follows:

32. (Thrice Amended) A method for producing a transgenic [animal] non-human mammal, comprising introducing a cell comprising a satellite artificial chromosome into a female non-human mammal [animal]; and

allowing the cell to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.

33. (Twice Amended) The method of claim 32, wherein the cell is a mouse stem cell.

43. (Thrice Amended) A method of producing a transgenic non-human mammal [animal], comprising:

introducing nucleic acid into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises a neo-centromere, the nucleic acid and euchromatin;

transferring the minichromosome into a second cell, wherein the second cell is an animal cell;

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introducing the cell comprising the minichromosome into a female non-human mammal [animal]; and

allowing the cell introduced into the female animal to develop into a transgenic non-human mammal [animal] comprising a minichromosome; wherein,

the nucleic acid comprises DNA encoding a selectable marker and a gene product or products; and

the DNA encoding the selectable marker and the DNA encoding the gene product or products are introduced into the cell simultaneously or separately.

44. (Thrice Amended) A method of producing a transgenic non-human mammal [animal], comprising:

introducing a nucleic acid fragment into a cell, wherein the nucleic acid fragment comprises a selectable marker;

growing the cell under selective conditions to produce cells that have incorporated the nucleic acid fragment into their genomic DNA;

selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises a neocentromere, the selectable marker and euchromatin;

introducing into the cell DNA encoding a gene product or products;

growing the cell under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are produced;

isolating the minichromosome and introducing it into an animal cell;

introducing the cell comprising the minichromosome into a female non-human mammal [animal]; and

allowing the cell introduced into the female [animal] mammal to develop into a transgenic non-human mammal [animal] comprising a minichromosome.

71. (Amended) The method of claim 43, wherein the animal cell is [an] a mouse embryonic stem cell or a fertilized ovum.

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73. (Twice Amended) A method for producing a transgenic non-human mammal [animal], comprising:

introducing DNA encoding a gene product or products into a cell containing the minichromosome of cell line EC3/7C5;

growing the cell under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are produced;

isolating the minichromosome and introducing it into an animal cell;

introducing the cell comprising the minichromosome into a female non-human mammal [animal]; and

allowing the cell introduced into the female mammal [animal] to develop into a transgenic non-human mammal [animal] comprising a minichromosome.

74. (Twice Amended) A method for producing a transgenic non-human mammal [animal], comprising:

introducing DNA encoding a gene product or products into a cell containing the  $\lambda$  neo-chromosome of cell line KE1 2/4;

growing the cell under selective conditions, whereby cells comprising the  $\lambda$  neo-chromosome comprising the DNA encoding the gene product(s) are produced;

isolating the  $\lambda$  neo-chromosome and introducing it into an animal cell;

introducing the cell comprising the minichromosome into a female non-human mammal [animal]; and

allowing the cell introduced into the female mammal [animal] to develop into a transgenic non-human mammal [animal] comprising a minichromosome.

93. (Amended) A method of producing a transgenic non-human mammal [animal], comprising:

introducing nucleic acid comprising a selectable marker into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

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selecting a cell comprising a satellite artificial chromosome;

transferring the satellite artificial chromosome into a second cell, wherein the second cell is an animal cell;

introducing the second cell comprising the satellite artificial chromosome into a female non-human mammal [animal]; and

allowing the cell to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.

95. (Amended) A method of producing a transgenic non-human mammal [animal], comprising:

introducing nucleic acid comprising a selectable marker into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

selecting a cell comprising a dicentric chromosome that comprises a *de novo* centromere;

growing the cell under conditions whereby a satellite artificial chromosome is produced;

transferring the satellite artificial chromosome into a second cell, wherein the second cell is an animal cell;

introducing the second cell comprising the satellite artificial chromosome into a female non-human mammal [animal]; and

allowing the cell to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.

96. (Amended) A method of producing a transgenic non-human mammal [animal], comprising:

introducing nucleic acid comprising a selectable marker into a first cell;

growing the cell under conditions that selectively permit the growth of cells containing the nucleic acid;

selecting a cell comprising an artificial chromosome that comprises more heterochromatic nucleic acid than euchromatic nucleic acid;

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transferring the artificial chromosome into a second cell, wherein the second cell is an animal cell;

introducing the second cell comprising the artificial chromosome into a female non-human mammal [animal]; and

allowing the cell to develop into a transgenic non-human mammal [animal] comprising an artificial chromosome that comprises more heterochromatic than euchromatic nucleic acid.

97. (Amended) A method for producing a transgenic non-human mammal [animal], comprising:

introducing an embryo comprising a satellite artificial chromosome into a female non-human mammal [animal]; and

allowing the embryo to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.

98. (Amended) A method for producing a transgenic non-human mammal [animal], comprising:

introducing a fertilized oocyte comprising a satellite artificial chromosome into a female non-human mammal [animal]; and

allowing the embryo to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.

99. (Amended) A method for producing a transgenic non-human mammal [animal], comprising:

introducing a mouse embryonic stem cell comprising a satellite artificial chromosome into an embryo;

introducing the embryo into a female non-human mammal [animal]; and

allowing the embryo to develop into a transgenic non-human mammal [animal] comprising a satellite artificial chromosome.